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An updated view of hypothalamic–vascular–pituitary unit function and plasticity

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Abstract

The discoveries of novel functional adaptations of the hypothalamus and anterior pituitary gland for physiological regulation have transformed our understanding of their interaction. The activity of a small proportion of hypothalamic neurons can control complex hormonal signalling, which is disconnected from a simple stimulus and subsequent hormone secretion relationship and is dependent on physiological status. The interrelationship of the terminals of hypothalamic neurons and pituitary cells with the vasculature has an important role in determining the pattern of neurohormone exposure. Cell in the pituitary gland form networks with distinct organizational motifs that are related to the duration and pattern of output. modification of these occurs in different physiological states, can persist after cessation of demand and results in enhanced function. Consequently, the hypothalamus and pituitary can no longer be considered as having a simple stratified relationship: with the vasculature they form a tripartite system, which must function in concert for appropriate hypothalamic regulation of physiological processes, for example reproduction. An improved understanding of the mechanisms underlying these regulatory features has implications for current and future therapies that correct defects in the hypothalamic–pituitary axes. In addition, recapitulating proper network organization will be an important challenge for regenerative stem cell treatment.

[H1] Introduction

To maximize reproductive success, via the appropriate timing of ovulation, lactation, or body growth, the outputs of several hypothalamic–pituitary axes are dramatically altered. These adaptive changes occur over differing time scales, with varying frequencies and levels of predictability. For example, the occurrence of the increase in growth hormone (GH) output at puberty is largely predictable. On a relatively short time scale (days), the surge in luteinizing hormone (LH) secretion required for oestrus is an acute change that occurs regularly once every reproductive cycle and, in humans, continues for years in the absence of pregnancy. On a longer time scale (months to years depending on the species), the increase in prolactin required for lactation is maintained for a variable time (which depends on when offspring are weaned) and recurs at each pregnancy, but is unpredictable before gestation. These large changes in the output of the various pituitary axes require modification of both hypothalamic and pituitary function, but whether this effect is reversed on cessation of physiological demand likely reflects the expectation that increased output will recur. A mechanistic understanding of these alterations in hypothalamic–pituitary function is fundamental to interpret and treat defects that lead to endocrine diseases resulting from hormone deficiencies (for example, dwarfism) or excess (such as polycystic ovarian syndrome). In this Review, we will focus on three pituitary axes that have roles in driving changes in physiology; the gonadatropin, prolactin and GH axes. The level of our understanding varies for each of these axes and the features that might serve as general principles of hypothalamic-pituitary and other endocrine organ function will be highlighted in the text.

[H1] Beyond stimulus-secretion coupling

The path of communication between the brain and peripheral tissues is exemplified by muscle contraction, which requires the transfer of electrical signals from axons via the neuromuscular junction. This sequence of events, known as excitation-contraction coupling[1], takes <1 s in mammals and adapts to altered functional demand. Similarly, in the hypothalamus just a few thousand neurons can also send signals to the periphery, in this case towards the median eminence (ME) via a specialized neurohaemal junction. In this junction, nerve terminal depolarization, either originating from the perikarya[2] or the terminals themselves[3], allows the sufficiently rapid entry of calcium ions to trigger exocytosis of neurohormones towards the first

loop of the portal fenestrated capillaries[4,5]. This rapid (<1 s) sequence of events was termed 'stimulus-secretion coupling' due to the clear similarities with excitation-contraction coupling[5,6]. Soon after release, neurohormones pervade the second loop of fenestrated capillaries within the pituitary gland, before binding to cognate receptors on endocrine cells to induce pituitary hormone exocytosis through a second 'stimulus-secretion coupling' event (FIG. 1)[7-10].

In the second half of the twentieth century (and building on Harris' work on the hypothalamus–pituitary axis[11]) the analogy between excitation-contraction and stimulus-secretion coupling was developed further[12]. However, important and fundamental differences exist between the two processes. Specifically, in the hypothalamus, endogenous rhythms with axis-dependent frequencies exist and the time scale for pituitary hormone release is measured in minutes to several hours[13]. Nevertheless, the analogy with neural control of locomotor activity led to a generally accepted model of hypothalamic regulation of pituitary function (FIG. 1).. Specifically, the excitation of specific hypothalamic neuron populations, determined by higher brain centres and peripheral feedback, is relayed as an unmodified series of signals to drive balanced pituitary hormone output[11]. The release of neurohormones and subsequent transportation and the effects on target cells were considered to be passive events in the regulation of pituitary hormone secretion, with only variation in the number of endocrine cells seeming to affect response levels[11,14]. Similarly, the alterations in gene expression and cell proliferation, which support maintenance of hormone output, were simply considered a correlated response to hypothalamic regulation of secretion[15].

In the early twenty-first century, a series of paradigm shifts in our understanding of the hypothalamic–pituitary system was established as a consequence of newly developed tools and techniques, including calcium imaging, fluorescent protein identification of specific cell types and two-photon microscopy, for use in genetically modified mice (eg[16-18]). The use of these methods have shown that both the pituitary gland and portal system can no longer be considered as static structures simply responding to neurohormonal regulation (BOX 1), although they have confirmed the pre-eminence of the hypothalamus as the driver of pituitary hormone output. In addition, hypothalamic neuron function has been found to be more dynamic than

initially thought, which might contribute to modifications in its regulation of the pituitary under different physiological states (FIG. 1)[2].

[H1] Gonadotroph axis

The reproductive system is critically dependent upon pulsatile secretion of gonadotrophin-releasing hormone (GnRH) and LH; however, the understanding of pulse generation has been hampered by the complexity of the regulatory mechanisms, many of which will clearly be lost in *in vitro* preparations. Investigators working in the late 1980s using pituitary portal bleeding and microdialysis documented the pulsatile nature of GnRH release into the portal vasculature of the sheep, monkey and rat[19-25], and showed a strong correlation between GnRH and LH pulses[22,26,27]. However, the scattered distribution and relative paucity of GnRH cell bodies limited the investigation of the cellular events that lead to pulsatile secretion of LH *in vivo*. In the past few years, the development of optogenetic techniques in rats and mice and an ultrasensitive ELISA capable of measuring LH levels in whole blood microsamples[28] has enabled investigators to dissect the GnRH neuron excitation parameters that generate LH pulses[29]. In these studies, the stimulation of just 60 GnRH neurons (~5% of the GnRH neurons) can trigger short-lived increases in LH secretion that resemble endogenous pulses[29]. Given the critical importance of GnRH neurons to the survival of all mammalian species, a degree of functional redundancy within this cell population is expected. This finding is consistent with studies in which just 10% of the GnRH neuron population is sufficient to maintain pulsatile LH secretion[30,31]. Varying the timing and frequency of stimulation has demonstrated that a brief (2 min) optogenetic stimulation at high frequency (10 Hz) evokes an LH pulse, whereas shorter periods and lower frequencies cannot elicit LH output that resembles endogenous pulses[29]. A bursting pattern of stimulation, which had been assumed to be most effective for pulse generation and the focus of many previous studies[32], also failed to increase LH secretion. Whether such a stimulatory signal exists endogenously *in vivo* and where its origin might be is unknown, although a 'GnRH pulse driver' might be located in the mediobasal–hypothalamus, specifically at the level of neurons co-expressing kisspeptin, neurokinin B, as well as dynorphin A (so-called 'KNDy' neurons)[33,34].

The generation of GnRH pulses, requires a coordinated release from multiple neurons through the synchronization of GnRH neuron population excitation. The cell bodies

of GnRH neurons are scattered throughout the basal forebrain, while their projections have dendrodendritic bundling and/or shared synapses, and become highly concentrated around the ME[35]. Fascinatingly, these projections simultaneously receive and integrate synaptic inputs — they possess both axonal and dendritic characteristics, leading to their description as 'dendrons', before finally acquiring an axonal morphology within the ME and ramifying into numerous terminals that appose blood vessels[36]. Dendrons might be an ideal location for putative afferent axons to modulate the excitability of multiple GnRH neuron dendrites, and for multiple GnRH neurons to align their firing pattern, which thereby provides a potential mechanism for their synchronized activity directly in the mediobasal hypothalamus[36]. An additional source of pulse synchronization is in the ME, where hypophysiotropic GnRH neurons terminate within the external zone close to endothelial cells of the portal vasculature[37]. Endothelial cells in the ME might modulate GnRH release through nitric oxide secretion[38-40] (reviewed elsewhere[41]). At the ME, nitric oxide is spontaneously released from an endothelial source and follows a pulsatile and cyclic pattern of secretion[38], and inhibition of nitric oxide synthesis within the ME can disrupt reproductive cyclicity[39]. Conversely, in the GnRH neuron perikarya, basal nitric oxide synthase activity might provide the tonic inhibition of the GnRH neural system required to maintain nadir levels of LH[40].

Once released into the ME, the transport of GnRH to the pituitary, and the pattern of gonadotroph exposure to the neurohormone, have been largely assumed to represent a simple linear process[22]. However, the use of fluorescent tracking using 4 kDa dextran, which mimics the size of most hypothalamic neurohormones, has shown that the diffusion processes, both at the level of the ME and the pituitary capillaries, are complex and non-linear[8]. Consequently, the portal vessel network might function as a 'physical integrator', enabling neurohormones to be transferred from the ME to the gonadotroph within a few seconds[42]. Once in the blood stream, the moderately rapid clearance rate (which varies between species) of LH generates the specific asymmetric pulse shape of this hormone, which is characterized by a fast increase immediately followed by a slower decrease[28]. Importantly, a faithful delivery of the pulsatile pattern of GnRH secretion to the pituitary is crucial for gonadotroph function[43-46]. For example, high GnRH pulse frequencies (>1 pulse per h) activate LH production, whereas low frequencies (<1 pulse per 2–3 h) preferentially induce

follicle-stimulating hormone (FSH) synthesis and release[47]. Overall, the intricate relationships between pulsatile GnRH release, secretory competency of the pituitary gonadotrophs and regulatory mechanisms within the vasculature generate the rhythmic fluctuations in LH secretion.

[H3] GnRH and LH surge generation

The GnRH/LH surge mechanism is sex specific and normally occurs only in women[48,49]. During the oestrous cycle, increasing concentrations of plasma oestrogen alter feedback to the GnRH neuronal afferent networks and gonadotrophs from negative to positive to induce the gonadotrophin surge[50-52]. That the oestrogen-responsive kisspeptin neurons in the rostral periventricular area of the third ventricle have a critical role in enabling ovulation in rodents by activating GnRH neurons is now well accepted[53]. Importantly, the relative contribution of the hypothalamic and pituitary levels to the oestrogen-induced gonadotropin surge seems to be species-dependent, with the latter the predominant mechanism in human and non-human primates[54]. In the female sheep, the GnRH surge is composed of high-frequency pulsatile events superimposed on a constantly elevated level of GnRH release, although whether the surge is driven by a fundamentally altered pattern of GnRH secretion[55], or by a simple increase in the frequency of pulsatile secretion is unclear[56]. This huge increase in GnRH secretion continues for a period of 24 h, considerably longer that the duration of the LH surge it induces, before returning to a strictly episodic pattern of release[25,55,57]. The firing pattern of GnRH neurons needed to generate the GnRH/LH surge is unknown. However, it is reasonable to assume that the prolonged firing of an increased number of GnRH neurons is required for the secretion of surge levels, compared with that required for a pulse. Indeed, in transgenic mice with a dose-dependent reduction in GnRH neuron migration, 10% of the normal GnRH neuronal content failed to rescue ovulation, but cyclicity was restored when approximately 30% of the GnRH population was present[31].

In addition to the putative change in GnRH population electrical activity, anatomical changes are found within the external zone of the ME where GnRH nerve terminals are ensheathed by tanycytes[58,59] (Fig. 2) The cellular conformation changes with fluctuating oestrogen profiles throughout the oestrous cycle. For example, in rats, semaphorin-7a-dependent structural remodelling of tanycytes occurs during the

preovulatory surge, resulting in release of the engulfed axons and direct access of GnRH nerve terminals to the portal vasculature[60] (FIG. 2). By contrast, fenestrated rat endothelial cells of the hypothalamic–hypophyseal portal vessels release semaphorin-3A, which is thought to induce GnRH neuron axonal growth and sprouting within the ME as a function of the oestrous cycle[61]. These mechanisms are likely to enable the generation of high concentrations of GnRH, which evoke the GnRH/LH surge, to be released into the pituitary portal circulation[62,63].

Within the pituitary, the distinct network organization of gonadotrophs[64] and their large scale reorganization during puberty[65] suggests that communication mediated by cell contact between gonadotrophs might have a functional role in the regulation of gonadotrophin output (Box 1). However, this organisation has not been studied in the same detail as that of other pituitary axes to date. Although the dynamic gonadotroph responses at the time of the proestrous surge have not yet been described *in vivo*, sampling of pituitaries at single time points in rats and sheep suggest that changes occur in gonadotrophin subunit expression, granule distribution and GnRH receptor abundance[66,67]. Sequencing of mRNA isolated from gonadotrophs of the anterior pituitary glands from female mice reveals that genes regulating the secretory process, blood pressure and cell adhesion were also enriched during proestrus[68]. Likewise, immortalized cell lines and cells in pituitary slices increase their cellular movement following GnRH stimulation[69], and extend cellular processes and increase their cellular movement at puberty[65] (FIG 3 and Box 1). These findings suggest that changes in the relationship of the gonadotroph network with the vasculature might modify the secretory response of gonadotrophs[70].

[H3] Clinical relevance

The mechanisms that underlie both pulsatile secretion and surge generation of LH have important implications for the treatment of infertility in women. For example, polycystic ovarian syndrome (PCOS), the most common anovulatory cause of infertility[71] affecting >100 million women worldwide, is associated with a dysregulation of the normal pattern of LH secretion[72]. Whether the origin of this multi-factorial disorder is at the level of the hypothalamic–pituitary axis is unknown[73], but PCOS is characterized by increases in GnRH pulse frequency and sensitivity of the pituitary gland to the neurohormone[74,75]. Consequently, potential interventions that modify the dynamics of GnRH output, its transport to the ME or its

actions in the pituitary might have implications for the treatment of PCOS. This is also the case for congenital hypogonadotropic hypogonadism, which results from a pituitary or a hypothalamic defect with or without anosmia[76]. Several novel gene mutations that are associated with this disorder have been identified, including those encoding neuropeptides (such as kisspeptin), transcription factors (chromodomain helicase DNA-binding 7) and G-protein coupled receptors (GnRHR)[77]. To induce female fertility, hypogonadotropic hypogonadism of pituitary origin can be reversed by subcutaneous injections of FSH followed by human chorionic gonadotrophin or LH to trigger ovulation[76]. Conversely, hypogonadotropic hypogonadism of hypothalamic origin can be treated using GnRH pumps to restore pituitary hormone secretion[76]. Pulsatile GnRH has the advantage of decreasing the risk of multiple pregnancy and ovarian hyperstimulation syndrome[78]. In both situations, the pulsatility of GnRH or the rhythmic secretion of LH and/or FSH is required to obtain sufficient follicular maturation and proper ovulation[76]. Advances in understanding of GnRH secretion and its interactions with LH are essential for designing novel, and indeed modifying existing, therapies for hypogonadotropic hypogonadism. For example, an estimated 22% of patients with this disease, who are yet to undergo treatment, have transient phases of normal fertility[79]. The underlying mechanisms and relevant therapeutic interventions to maintain this phenomenon might be elucidated by further investigation of pulsatility and rhythmicity.

[H1] The prolactin axis

The prolactin axis is unique among the pituitary hormonal systems, as in men and non-lactating women it can be considered a system primed for activation but tonically inhibited by hypothalamic dopamine[80]. In this situation, low concentrations (<25 ng/ml) of circulating prolactin are maintained by short-loop feedback, with prolactin receptor-mediated stimulation of dopamine neuron firing rate leading to an increase in catecholamine production[81] and output[2]. The timescale of the feedback response to prolactin (~10–20 min) could be explained by coordinated release of dopamine from multiple neurons[2]. One possible mechanism is the coordinated changes in firing rates of a subset of tuberoinfundibular dopamine neurons over tens of minutes; these correlate with episodic dopamine secretion recorded from multiple terminals at the median eminence in mice[2]. Gap junctions and local dendritic dopamine release have

been proposed to mediate this activity[82,83], and integration of single cell firing rates seems to be involved in the generation of longer dopamine release output events (N. Romano and P. Mollard, unpublished data).

Variations in prolactin output occur in virgin female rats as a surge at proestrus, which coincides with that of LH[84]. Prolactin also increases following vaginal stimulation of both rats[85] and mice[86] as twice daily surges. These surges are coordinated by signals from the suprachiasmatic nucleus[87], most likely through the actions of vasoactive-intestinal peptide[88]. At the level of the pituitary, lactotrophs form a network of honeycomb motifs (Box 1) that allow the congregation of cells along the fine pituitary capillary network (FIG. 3)[89]. This organization supports low levels of cell-cell coordination, with a small proportion (~1–10%) of cells acting as coordinating nodes by functionally connecting distant ensembles[90]. In addition to synchronizing Ca^{2+} activity, cellular organization also mediates the coordination of gene transcription, with gap junction signalling enabling local correlation of bursts of transcriptional activity that are otherwise randomly timed[91,92]. This mechanism resembles quorum-sensing where apparently random systems display complex activity as long as the components (in this case the cells) can interact, and might contribute to hormone gene expression and cell proliferation[92-94]. Precisely how gap junctions might orchestrate this mechanism remains unknown.

[H3] Increased prolactin output during lactation

The long-term requirement for large increases of circulating prolactin in lactation is associated with a decrease in dopamine output, which begins in late pregnancy and is coincident with a surge of pituitary prolactin secretion[95] (FIG. 2). The dopamine tone needs to be strongly decreased throughout lactation to enable the necessary increase in circulating prolactin, and is mediated by a decrease in phosphorylation of tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis[95]. This mechanism is not the result of a reduced feedback of prolactin on dopamine neurons, which remain electrically responsive at the level of the cell body, but rather, neuronal firing becomes uncoupled from dopamine secretion[2]. Remarkably, the reduction in dopamine tone is accompanied by the production of opioids, which might enable these neurons to stimulate prolactin secretion[96,97].

In concert with changes in the hypothalamic inhibition of prolactin secretion, substantial alterations occur in the pituitary to support the 10–50-fold increase in prolactin secretion that is required for milk production in mammals[98]. In humans and rats, this increased hormone secretion is generally accompanied by proliferation and hypertrophy of lactotrophs, although the studies describing hypertrophy are based on 2D histological studies[98]. By contrast, in lineage tracing and FACS studies in mice, lactotrophs become hypertrophied during lactation and increase their volume threefold without the accompanying increase in number[99]. Other investigators have confirmed these findings, and also showed that the lactotroph network *in situ* becomes highly-connected during lactation, which is associated with the strength of the suckling stimulus[100]. This increase in structural connectivity leads to an ~100% increase in the proportion of the subpopulation of lactotrophs that function as coordinating nodes and orchestrate increased output of prolactin[100].

[H3] Memory of prolactin demand after weaning

At weaning, a rapid decrease in prolactin secretion occurs as a result of a return of dopamine inhibition[101]. In rodents[101,102] and humans[103] basal prolactin secretion is reduced below that of virgin animals, which might reflect an enhanced pituitary response to dopamine inhibition[104]. Strikingly, and despite this reduction in basal prolactin secretion, lactotrophs remain enlarged and well-connected with each other at both the structural and functional levels, with a twofold increased number of nodes, which persists for many months after lactation has ceased[100]. Such hard-wiring or 'memory' of previous stimuli, which was previously thought to only exist for neurons and immune cells, leads to augmented network-mediated lactotroph calcium activity during suckling of subsequent litters, which drives even higher concentrations of prolactin[100]. This mechanism is independent of reproductive experience *per se*, since it can be prevented by reducing the suckling demand[100].

[H3] Clinical relevance

The dysregulation of the prolactin axis, owing to either pituitary adenomas[105] or as an adverse effect of treatment with antipsychotic drugs[106], leads to impaired fertility. Hyperprolactinaemia has a prevalence of ~10 to 30 per 100,000 in men and women, respectively, and is the second most common cause of infertility in women after PCOS[107]. Whilst treatment with commonly-used dopamine receptor agonists is an

effective treatment of the majority of hyperprolactinaemic patients, side effects of these drugs including nausea, headaches and postural hypotension leads to compliance problems[108]. Clearly, an understanding of the interactions that lead to altered dopamine output and the response of the pituitary might help to identify novel treatment strategies for this disease. In rodent studies, prolactin seems to affect multiple neuroendocrine axes, including those regulating fertility, body weight and appetite, stress and maternal behaviour[80] and these warrant further study to determine the potential effects of its over-secretion in humans. For example, hyperprolactinaemia might lead to changes in GnRH neuron activity via interactions with the GPR54/kisspeptin pathway in mice[109] and GnRH pulsatility has been reinstated in mice with physiological hyperprolactinaemia by administration of kisspeptin[110]. By contrast with the study in mice where an acute high dose of prolactin was delivered peripherally[109], in studies treating sheep with a chronic centrally administered low (10,000-fold lower) prolactin dose, no effects of prolactin on hypothalamic kisspeptin expression have been seen[111]. An improved understanding of these pathways could aid the development of treatments for women with hyperprolactinaemia that is resistant to dopamine agonists[108].

[H1] The GH axis

In humans and animals, such as cattle and horses, in which the measurements can be taken, pulsatile GH output is present from birth[112-114]. However, the output is markedly increased at puberty when sexually-dimorphic body growth occurs[115]. Since the discoveries of GH releasing hormone (GHRH) and somatostatin that control GH secretion from pituitary somatotrophs[115-117], a remarkable advancement our understanding of GH pulse generation during critical physiological windows has taken place.

[H3] Pulsatile GHRH output

Using genetically-modified mouse models with GHRH neurons marked with green fluorescent protein[118], several investigators have defined the mechanisms that underlie pulse generation using *ex vivo* slices of brain. Before puberty, GHRH neurons are excitable neuroendocrine neurons with complex synaptic inputs[119]. These early stages of hypothalamic development ensure appropriate regulation of the somatotroph axis, as shown in the Ames dwarf mice in which loss of GH leads to a compensatory

increase in GHRH cell number[120]. Additionally, steroid exposure in young animals can have programming effects on the GHRH neuron population, with testosterone exposure in neonates permanently increasing adult GHRH cell number and GHRH gene transcription[121]. Modification of these synaptic inputs and electrical properties over the first 6 weeks of postnatal life correlates with and likely drives, at least in part, increased pituitary GH output and sexual dimorphism[122]. The intrinsic hourly rhythms of GHRH neuronal activity predicted by simulation studies of *in vivo* GH pulsatility have not been indentified[119,123], however, somatostatin can generate GHRH neuron pulsatile output by delaying oscillations of action potential firing via a recurring inhibition of inhibitory GABAergic interneurons (that is, inhibition of inhibition)[123]. Consequently, somatostatin can both acutely inhibit the excitability of GHRH neurons and also promote their patterned output together with more sustained GHRH neuron stimulation in response to other stimuli in the brain (for example, acetylcholine[122]) and peripheral tissues (such as, ghrelin)[117].

[H3] Modification of pituitary somatotroph output

No full description of the *in vivo* dynamics of GHRH and somatostatin neurons and their regulation of pituitary somatotrophs exist. This event can be viewed as a three-step process: delivery of neurohormone to target cells; cellular secretory responses to regulation; and entry of pituitary hormone into the peripheral circulation[8]. *In vivo* imaging of the mouse portal system and somatotroph network have provided insights into the first step in this process and the role of the vasculature in shaping the pattern of exposure of the pituitary to hypothalamic neuropeptides[8]. In this study, delivery of neuropeptides such as GHRH to the somatotroph network, which extends throughout the pituitary gland, follows specific vascular/capillary routes and results in specific temporal patterned regional regulation rather than a homogenous exposure of the whole pituitary to the secretagogue. In addition, the initial stimulation by GHRH evokes a coordinated enhancement of oxygen supply to the stimulated somatotroph network via increased capillary blood flow, which provides fuel for energy-depleting secretory responses. Indeed, this study also demonstrated that capillaries closely line the clusters and strings of cells that form the GH cell, which suggests an important role of local oxygen regulation on GH release (Figure 3). The dynamic association of gonadotrophs with the vasculature, which varies through the ovarian cycle[65], suggests that this situation might also be the case for other pituitary axes. The second step in

pituitary regulation has been characterized using *ex vivo* data from acute pituitary slices where the somatotroph network organization is preserved, in which the homotypic network mediates coordination of stimulation, triggering long-lasting GH secretion[124] (Box 1) These studies have also shown that network organization is likely to have a major role in the increased GH output at puberty. In particular, using males the study showed that the volume of the GH network undergoes large changes relative to its surface area that coincides with the onset of puberty, before gradually returning to normal prepubertal levels by day 100 in mice[16]. These changes occur with a timing that is coincident with the increased pulse output of GH associated with puberty in males and are blocked by castration, which also prevents pubertal changes in GH output[16]. A direct effect of sex steroids on organisation of pituitary cells into homotypic networks is apparent from the rapid and dramatic increased motility of somatotrophs in *ex* vivo pituitary cultures treated with oestradiol[125]. These findings highlight the importance of somatotroph network organization and its plasticity in the generation of pituitary somatotroph output. The vasculature also has an important role in somatotroph output, where *in vivo* imaging shows that capture of secreted GH is a controlled event where the perivascular space acts as a gate-keeper for hormone entry into the capillary lumen[8]. The relationship between cellular network organization and the vasculature in the pituitary is, therefore, central to the delivery of incoming hypothalamic signals, and the build-up of GH pulses within capillaries.

[H3] Clinical relevance

GH deficiency resulting from congenital defects or acquired following traumatic brain injury, pituitary tumours or cranial irradiation[126,127], is commonly treated with a daily subcutaneous dose of recombinant GH in childhood to increase growth rate[128]. However, considerable uncertainty exists regarding the optimal dosage or regime, and current treatments by injection of GH do not fully recapitulate the physiological pattern of GH secretion[115]. One potential therapy is repopulation of the pituitary with stem cells, which have been identified in the mouse[129]. However, such approaches are likely to require the recapitulation of the normal cellular organization to achieve normal pulsatility and homeostatic regulation.

Patients with acromegaly, which results from a GH secreting pituitary adenoma, frequently have glycaemic disorders: a lack of GH pulsatility modifies lipolysis,

whereas overall GH hypersecretion can induce insulin resistance[130]. Consequently, an improved understanding of the mechanisms that determine the pattern of GH output might help to define new therapeutic options for dyslipidaemia or diabetes mellitus. In addition, GH pulsatilty also has an important role in a subgroup of patients who have clinical acromegaly with increased insulin-like growth factor 1, but unaltered mean 24-h GH concentration compared with healthy controls[131,132]. Altered GH pulsatility might explain the clinical presentation of this sub-group of patients, and our new understanding of the mechanisms underlying patterning of pituitary output might explain the abnormal GH axis function in these individuals and warrants further investigation.

[H1] Conclusions

The examples in this Review provide new insights into regulation of three hypothalamic–pituitary axes and demonstrate that these mechanisms are not a simple relay of stimulus-secretion coupled events. The disconnection or modulation of hypothalamic excitation and neurohormone release, and an active role of the vasculature and pituitary in the network-mediated modification of responses, demonstrates that the previous stimulus-secretion coupled view of the hypothalamic–pituitary system is over-simplistic. Advances in imaging technologies are allowing us to understand more about the organization and function of this axis (FIG. 4). Given that the hypothalamus contains no more than a few thousand parvocellular neurons, the rapid development of techniques for interrogating neuronal function should enable the characterization of this structure's regulation and output. Such studies will be invaluable for the deeper understanding of mammalian physiology, as this region controls a much larger panel of known body functions than any other brain region.

The regulation of pulsatile pituitary secretion must now be considered as an integration of hypothalamic, vasculature and pituitary regulation, which has further implications for the understanding of disease. For example, the identification of kisspeptin has provided an exciting new target for the treatment of infertility[133]. The uncoupling of neuronal excitation and hormone output also has deeper implications such as in the case of ageing. For example, the reduction in GH output with age might be due to a failure of neurohormone secretion from GHRH neurons without any change in their excitation[129] (Figure 2). The human pituitary gland can be accessed by

transphenoidal surgery, which makes this structure an ideal target organ for regenerative therapy[134]. The pituitary cell networks and their relationship with the vasculature must be considered for such therapy and the microenvironment clearly has an important role in the regulation of the pituitary gland, which might also affect the development of tumours[135]. Finally, as pituitary networks are sensitive to peripheral regulation and their modification can persist for extended periods, they are a potential target for endocrine disrupting chemicals (EDCs). Indeed, the identification of bisphenol A-mediated reduction of expression of *ICAM5* in the pituitary[136] has led us to speculate that some EDC effects might be mediated by changes in pituitary organization[137]. These possibilities require further investigation for the understanding of both the aetiology and treatment of diseases associated with pituitary hormones.

Competing interests statement

The authors declare no competing interests

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Author contributions

The authors contributed equally to all aspects of the preparation of this article

Key points

- The activity of hypothalamic neurons is modified by inputs leading to heterogeneous activity; a small proportion of the total population can drive pituitary hormone pulsatility
- Neurohormone output can vary following neuron excitation according to physiological status, which might also lead to declining neuroendocrine output with age
- The release of hypothalamic factors into the blood is modified by alterations in the juxtaposition of nerve terminals with the vasculature and tanycytes in the median eminence
- Cells in the pituitary gland form homotypic networks, the organization of these relationships with the vasculature are distinct for each endocrine axis, which modifies responses to regulatory factors and patterns of output in response to demand
- Reorganisation of the pituitary network can store long-term memories of increased output and learn to increase function on repeated challenge
- Understanding the importance of coordinated hypothalamic–vasculature–pituitary function provides new understanding of a range of endocrine axes defects and targets for novel therapies

Figure 1 |A dynamic interplay between hypothalamic neuron output, the vasculature and the pituitary response alters hormone output. a) The concept of stimulus-secretion coupling considered pituitary hormone output to result from a cascade of events dictated by the pattern of regulatory neuron excitation. **b)** Recent studies have shown that this view (a) is too simplistic: alterations at the level of neuron response to excitation, release of hypothalamic factors in the median eminence and pituitary cell responses are all capable of adapting output of hormone to the periphery.

Figure 2 |Output of hypothalamic regulatory factors to the median eminence is mediated by a number of processes. a) A simplified view of tanycyte regulation of gonadotrophin releasing hormone (GnRH) release. Access of GnRH nerve terminals to capillaries at diestrus is blocked by tanycyte ensheathment, which retracts of at proetrus, possibly in response to oestrogen-driven secretion of nitric oxide (NO), leading to formation of a direct neurohaemal junction and augmented release of

GnRH. **b)** Feedback inhibition of prolactin secretion is reduced in lactation by an uncoupling of dopamine production and release from neuronal electrical activity and possible switch to production and secretion of opioids. **c)** Growth hormone releasing hormone (GHRH) secretion is reduced in late adulthood, with an altered localisation of nerve terminals and redistribution of secretory vesicles to autophagic vacuoles, both resulting in reduced neuropeptide release to the portal circulation.

Figure 3 Pituitary cells form homotypic networks, with distinct organisational motifs and relationship with the vasculature, which alter with physiological status. a) Gonadotroph cells are arranged as strings of cells which align with capillaries and make direct contact with the vasculature through protrusions at proestrus. **b)** The organisation of lactotroph into honeycomb structures in virgin mice becomes more pronounced in animals which are lactating, with an alteration of cell-cell contacts, increase in cell size, and appearance of cells which act as hubs of functional connectivity (dark orange). This change in structural and functional organisation is maintained for months following cessation of physiological demand at weaning. **c)** The homotypic network of somatotrophs changes dramatically at puberty, with the formation of large clusters which are linked by strands of cells in young males. The strands of cells found in juvenile and mature animals are closely associated with capillaries, which also line the post-pubertal somatotroph clusters

Figure 4 | Updated view of the hypothalamic–vascular–pituitary unit. In the past decade, a more complex relationship between the hypothalamus and pituitary than previously appreciated has emerged, in part owing to new imaging techniques which now allow high-resolution optical imaging of the hypothalamic-vascular-pituitary unit in living animal models. **a** | At the level of the parvocellular neurons, complex inputs modify the excitation of neurons, which can vary coupling with neurohormone output at terminals of the median eminence (ME) through modification of intracellular pathways. **b** | Alterations in tanycyte ensheathment and the anatomical location of neuron terminals modify their interactions with the vasculature, changing the dynamics of neurohormone release. **c** | In the pituitary gland, changes in blood flow in the portal circulation alter the pattern of exposure of pituitary cells to neurohormone and nutrient supply to facilitate secretion. **d** | Cells of the pituitary gland, such as somatotrophs, are organised into intermingled networks with distinct morphological features, which can be altered to meet physiological demand, and relationships with the vasculature.

Box 1 | Organization of pituitary cells into homotypic networks

Somatotrophs, corticotrophs, gonadotrophs and lactotrophs form networks with the following features[16,64,65,100,138,139]

- *The lineages have distinct developmental programmes*
    - Placement of each endocrine cell network occurs at distinct stages of pituitary organogenesis, before expansion in early postnatal life[16,64]
- *Networks have distinct motifs and interact with the vasculature*
    - Somatotrophs organise as clusters linked with strands[16] along the capillary network[138], while lactotrophs form a honeycomb structure[100] aligned with these vessels[138]. Gonadotrophs connect to one or more blood vessels via their protrusions[65], whereas the corticotroph network has a loose arrangement[64]. Cell-type specific homotypic network organisation and their distinct relationships with the vasculature are likely to affect levels and timing of hormone release [8, 16, 70, 100, 142].
    - The mechanisms underlying the distinct endocrine cell-vascular relationship are unclear, although one factor shown to be important is Prop1, since its loss leads to failure of organ vascularization[143]
- *Hypothalamic and steroidal factors generate network motifs*
    - Loss of growth hormone-releasing hormone (GHRH) leads to isolated somatotrophs, whereas somatotroph ablation with intact GHRH stimulation results in clusters of cells that are isolated from each other[140]. Gonadal steroids also influence somatotroph cell clustering and cell movement leading to reorganisation[124,125]
- *Pituitary networks have functional relevance*
    - Pituitary networks integrate, amplify and propagate hypothalamic signals that arrive from the median eminence. For example, the male somatotroph network responds to GHRH input with large, coordinated, oscillatory Ca^{2+} increases that outlast the stimulus to drive large excursions in hormone secretion[124].
- *Endocrine and non-endocrine homotypic networks interact*
    - Communication between each pituitary hormonal cell-type, as well as the non-hormonal folliculostellate cells, via gap-junctions mediated coupling[91,100] and paracrine and autocrine interactions[138,141], can modify output of each of the pituitary hormones and allow cross-talk between pituitary axes.
    - Loss of specific pituitary hormonal cell types influences the network organisation of heterotypic cells. For example, changes in the gonadotroph network occur following alteration of corticotroph terminal differentiation in mice[64].

Author biographies

Paul Le Tissier is a Senior Lecturer in the Centre for Integrative Physiology, University of Edinburgh, UK. His primary research interests are the understanding of hypothalamic regulation of the anterior pituitary gland, how this mechanism maintains and alters the function of the different cell populations to ensure appropriate output throughout life and modelling how dysregulation leads to pathology.

Pauline Campos has recently become a Postdoctoral Fellow in the laboratory of Patrice Mollard, at the Institute of Functional Genomics of Montpellier, France. She completed her PhD in the Centre for Neuroendocrinology at the University of Otago, New Zealand, and has been focused on developing cutting-edge tools to study the electrical activity of hypothalamic neurons underlying pituitary hormone pulsatility. Her current work aims at understanding the central control of thyroid function in conscious animals.

Chrystel Lafont is an INSERM Engineer in the Institute of Functional Genomics of Montpellier, France. She is the technical leader of a small animal *in vivo* imaging facility that develops cellular *in vivo* imaging techniques. Her research interests look at the role of the vasculature and pituitary cell networks in the build-up of hormone pulses.

Nicola Romanò is a Lecturer at the University of Edinburgh, UK. His main interest is the study of the cellular and systemic mechanisms of pulsatile hormone secretion in neuroendocrine axes, and how they correlate and influence physiological function. His research interests span from analysis of activity patterns of hypothalamic neurons to structural and functional analysis of pituitary cell networks, such as, the spatio-temporal analysis of cell activity.

David Hodson is a Professor in Cellular Metabolism at the University of Birmingham, UK. His laboratory is developing innovative optical methodologies that enable the mapping and manipulation of endocrine cells directly within their tissue context, focusing on the pituitary gland and pancreas as tractable systems.

Patrice Mollard is the Head of the Department of Physiology and a CNRS Research Director at the Institute of Functional Genomics, Montpellier, France. Dr Mollard's laboratory pioneered the study of large-scale functional organization of endocrine cell types within the pituitary gland in the early 2000s. His research is now exploring how pituitary cell networks function together with hypothalamic inputs and vasculature in health and disease.

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Fig 1

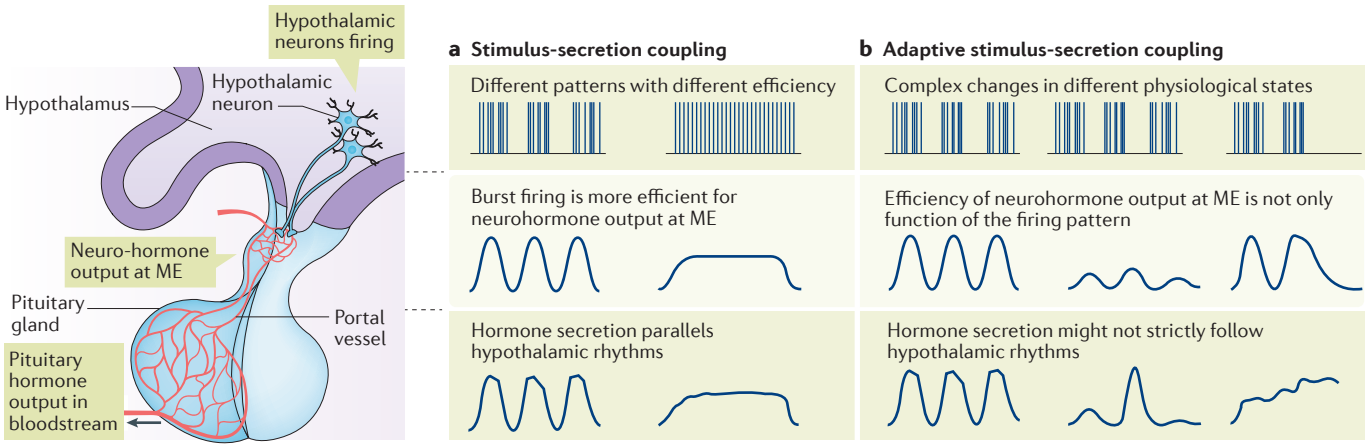

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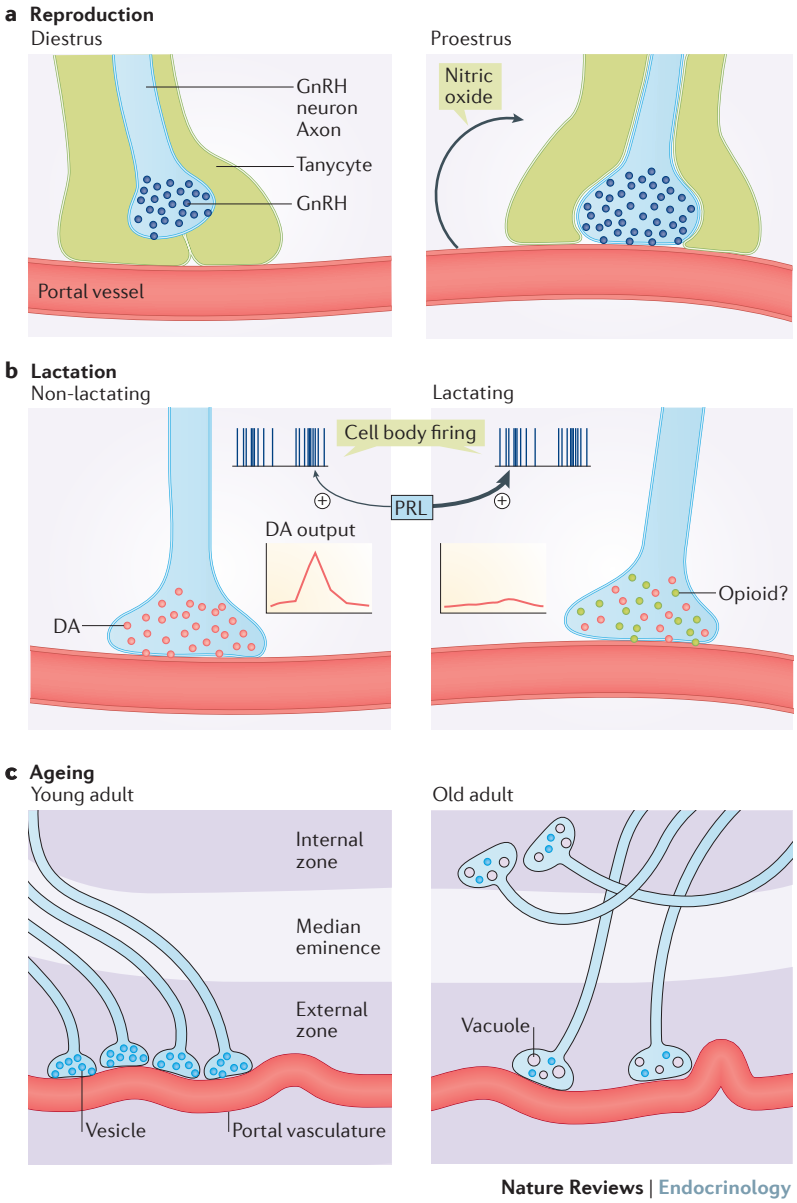
Fig 2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Vesicle
Portal vasculature
Vacuole
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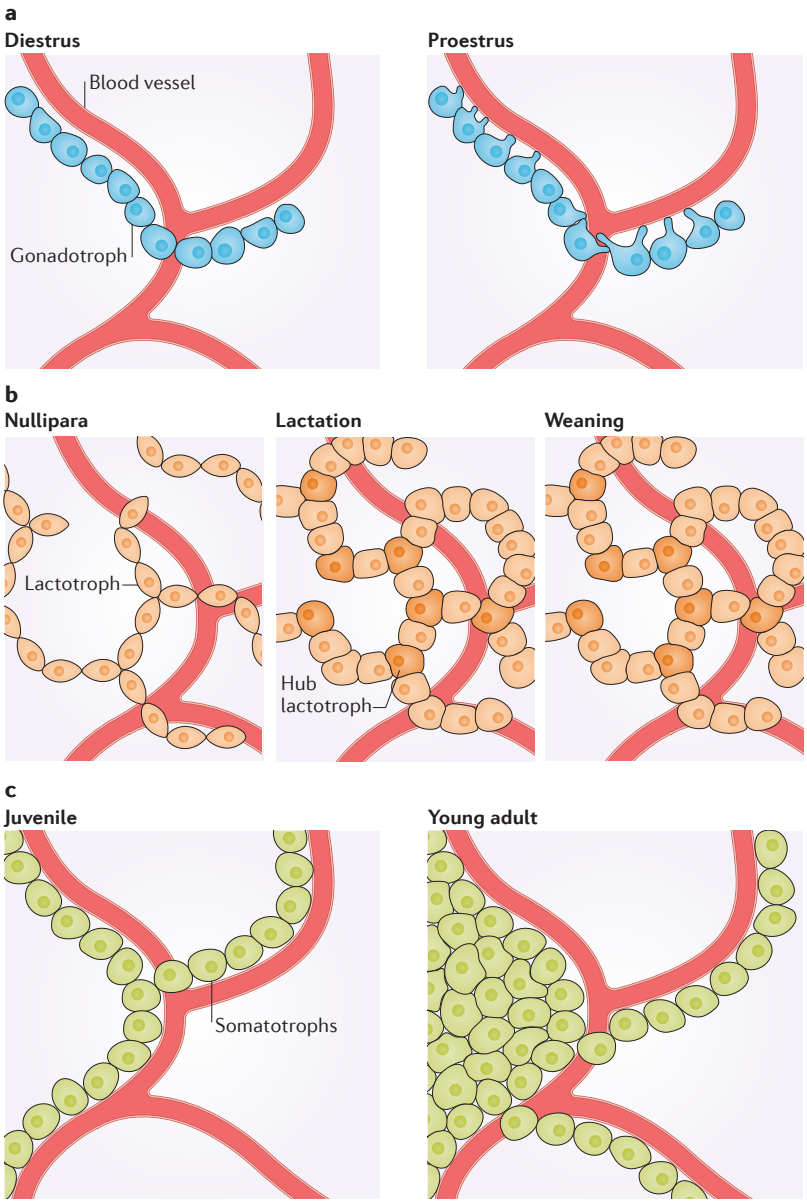
Fig 3


a
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Proestrus
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Hub lactotroph
Weaning
c
Juvenile
Somatotrophs
Young adult
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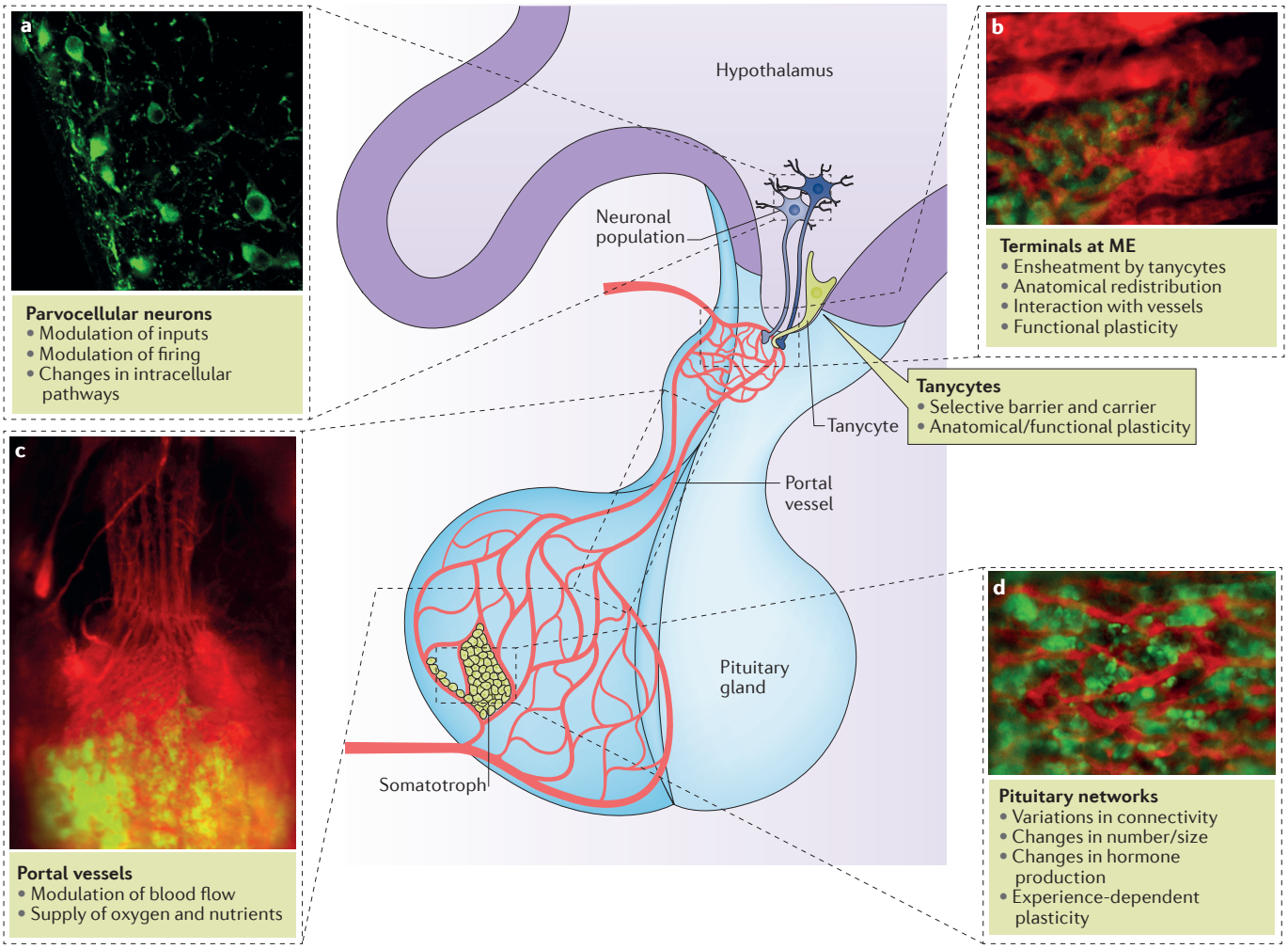
Fig 4


a
Parvocellular neurons
• Modulation of inputs
• Modulation of firing
• Changes in intracellular pathways
Hypothalamus
Neuronal population
b
Terminals at ME
• Ensheatment by tanycytes
• Anatomical redistribution
• Interaction with vessels
• Functional plasticity
Tanycytes
• Selective barrier and carrier
• Anatomical/functional plasticity
Tanycyte
Portal vessel
c
Portal vessels
• Modulation of blood flow
• Supply of oxygen and nutrients
Pituitary gland
Somatotroph
d
Pituitary networks
• Variations in connectivity
• Changes in number/size
• Changes in hormone production
• Experience-dependent plasticity